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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 76, Revision 1 (FGE.76Rev1)

Consideration of sulphur-containing heterocyclic compounds evaluated by JECFA (59th meeting) structurally related to thiazoles, thiophene, thiazoline and thienyl derivatives from chemical group 29 and miscellaneous substances from chemical group 30 evaluated by EFSA in FGE.21Rev3[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present opinion concerns a group of 26 sulphur-containing heterocyclic compounds evaluated by the JECFA at the 59th meeting in 2008. This revision is made due to the inclusion of one additional substance, 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004], cleared for genotoxicity concern in FGE.224. Additionally, new toxicity data have become available for 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113]. Since publication of FGE.76, one substance, thiazole [FL-no: 15.028], is no longer supported by Industry for use as a flavouring substance in Europe and will therefore not be considered any further. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel agrees with the application of the Procedure as performed by the JECFA for 21, [FL-no: 15.001, 15.002, 15.004, 15.008, 15.011, 15.013, 15.014, 15.015, 15.016, 15.017, 15.019, 15.020, 15.021, 15.022, 15.026, 15.027, 15.033, 15.035, 15.109, 15.113 and 16.027], of the 26 substances considered in this FGE and agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach. For five substances [FL-no: 15.005, 15.018, 15.029, 15.030 and 15.032], the Panel could not conclude on their safety when used as

[1] On request from the European Commission, Question No EFSA-Q-2013-00222, EFSA-Q-2013-00223, adopted on 24 October 2013.

[2] Panel members: Ulla Beckman Sundh, Mona-Lise Binderup, Claudia Bolognesi, Leon Brimer, Laurence Castle, Alessandro Di Domenico, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Martine Kolf-Clauw, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Kettil Svensson, Maria de Fatima Tavares Poças, Fidel Toldra and Detlef Wölfle. Correspondence: cef@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings: Ulla Beckman Sundh, Leon Brimer, Wilfried Bursch, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Trine Husøy, John Christian Larsen, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft, Pia Lund and Karin Nørby and EFSA staff: Annamaria Rossi and Kim Rygaard Nielsen for the support provided to this scientific opinion.

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flavouring substances, as these substances could not be evaluated because of concern with respect to genotoxicity. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered and for all 26 substances, the information is adequate.

KEY WORDS

flavouring safety, sulphur-containing heterocyclic compounds, thiazoles, thiophene, thiazoline and thienyl derivatives, 59th JECFA meeting, FGE.21

SUMMARY

Following a request from the European Commission, the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

In Flavouring Group Evaluation 76 (FGE.76), the EFSA considered 26 sulphur-containing heterocyclic compounds evaluated by the JECFA at its 59th meeting. Since publication of FGE.76, one substance, thiazole [FL-no: 15.028], is no longer supported by Industry for use as a flavouring substance in Europe and will therefore not be considered any further. The present revision is made due to inclusion of one additional substance, 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004], cleared for genotoxicity concern in FGE.224. Additionally, new toxicity data have become available for 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113]. Therefore, the present revision of FGE.76, FGE.76Rev1, considers 26 flavouring substances evaluated by the JECFA.

The Panel concluded that all 26 Register substances in the JECFA flavouring group of sulphur-containing heterocyclic compounds are structurally related to the 59 thiazoles, thiophenes, thiazoline and thienyl derivatives from chemical group 29 and miscellaneous substances from chemical group 30 evaluated by EFSA in the Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3).

In the previous version of FGE.76, the Panel considered that for the substances [FL-no: 15.109 and 15.113] no adequate NOAELs were available. Since then a 90-day study has become available for 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113] providing a NOAEL to establish adequate margins of safety for the substance, as well as for the structurally related 2,4,6-trimethyldihydro-1,3,5(4*H*)-dithiazine [FL-no: 15.109].

The Panel agrees with the application of the Procedure as performed by the JECFA for 21 of the 26 substances considered in this FGE. Three of the remaining five substances, 2-(sec-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029], 4,5-dimethyl-2-ethyl-3-thiazoline [FL-no: 15.030] and 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032], were considered by the Panel to have genotoxic potential *in vitro*, and therefore the Panel decided that the Procedure should not be applied to these three flavouring substances until adequate *in vivo* genotoxicity data become available. Additionally, the Panel noted the presence of a terminal conjugated double bond in the substances 2,4-dimethyl-5-vinylthiazole [FL-no: 15.005] and 4-methyl-5-vinylthiazole [FL-no: 15.018] which raised concern for genotoxicity. The Panel concluded that the Procedure should not be applied to these two substances either until additional data become available.

Thus, the Panel agreed that the Procedure can be applied to 21 of the 26 JECFA-evaluated substances [FL-no: 15.001, 15.002, 15.004, 15.008, 15.011, 15.013, 15.014, 15.015, 15.016, 15.017, 15.019, 15.020, 15.021, 15.022, 15.026, 15.027, 15.033, 15.035, 15.109, 15.113 and 16.027], whereas five substances [FL-no: 15.005, 15.018, 15.029, 15.030 and 15.032] cannot be evaluated using the Procedure until additional data become available.

For all 26 substances, the JECFA evaluation is based on MSDI values derived from production figures from the EU.

For all 21 substances evaluated through the Procedure use levels are needed to calculate the modified Theoretical Added Maximum Daily Intake (mTAMDI) in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 26 JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for all 26 the JECFA evaluated substances.

Thus, for five substances [FL-no: 15.005, 15.018, 15.029, 15.030 and 15.032] the Panel could not conclude on their safety when used as flavouring substances, as these substances could not be evaluated because of concern with respect to genotoxicity.

For the remaining 21 of the 26 JECFA-evaluated sulphur-containing heterocyclic compounds [FL-no: 15.001, 15.002, 15.004, 15.008, 15.011, 15.013, 15.014, 15.015, 15.016, 15.017, 15.019, 15.020, 15.021, 15.022, 15.026, 15.027, 15.033, 15.035, 15.109, 15.113 and 16.027] the Panel agrees with the JECFA conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[4] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[5]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

EFSA concluded that a genotoxic potential of the two α,β-unsaturated thiophenes in FGE.224 could not be ruled out. Information on 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] has now been submitted by the European Flavour Association.

The Commission asks EFSA to evaluate this new information and depending on the outcome proceed to the full evaluation of the flavouring substance in FGE.76.

EFSA concluded in FGE.76 that for two substances [FL-no: 15.109 and 15.113], there are insufficient data available to provide margins of safety from their use as flavouring substances and that additional toxicity data are needed.

The requested information on one representative material, 5,6-dihydro-2,4,6,tris(2-methylpropyl)-4*H*-l,3,5-dithiazine [FL-no: 15.113] has now been submitted by the European Flavour Association. This information is intended to cover the re-evaluation of this substance and of one substance from FGE.76 (2,4,6-trimethyldihydrol, 3,5(4*H*)-dithiazine [FL-no: 15.109]).

The Commission asks EFSA to evaluate this new information as well.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to carry out a safety assessment on 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004], 5,6-dihydro-2,4,6,tris(2-methylpropyl)-4*H*-l,3,5-dithiazine [FL-no: 15.113] and 2,4,6-trimethyldihydrol, 3,5(4*H*)-dithiazine [FL-no: 15.109] in accordance with Commission Regulation (EC) No 1565/2000.

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. Official Journal of the European Communities 31.12.2008, L 354/34-50.

[5] EC (European Commission), 2012. Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. Official Journal of the European Communities 2.10.2012, L 267, 1-161.OJ L 267, 2.10.2012, p. 1.

[6] Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000, hereafter named the "EFSA Procedure". This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996; JECFA, 1997; JECFA, 1999), hereafter named the "JECFA Procedure". The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be evaluated through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram (µg)/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the

Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 µg per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 µg per day?")" (JECFA, 1999).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 µg per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

1. HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE

The JECFA has evaluated a group of 30 flavouring substances consisting of sulphur-containing heterocyclic compounds (JECFA, 2002a).

In FGE.76, which covered a group of 26 of the 30 JECFA-evaluated substances, the Panel concluded that for six substances [FL-no: 15.005, 15.018, 15.028, 15.029, 15.030 and 15.032], the Procedure should not be applied until adequate genotoxicity data become available. For eight other substances, European exposure information (MSDI) was not available [FL-no: 15.002, 15.005, 15.008, 15.027, 15.029, 15.030, 15.109 and 15.113]. In addition, the Panel considered that for the substances [FL-no: 15.109 and 15.113], there were insufficient data available to provide margins of safety from their use as flavouring substances and that additional toxicity data were needed.

Since the publication of FGE.76, Industry has informed that thiazole [FL-no: 15.028] is no longer supported for use as flavouring substances in Europe (EFSA, 2011) and it will therefore not be considered any further.

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.76	31 January 2008	http://www.efsa.europa.eu/en/efsajournal/pub/875.htm	26
FGE.76Rev1	24 October 2013		26

The present revision of FGE.76, FGE.76Rev1, includes the consideration of one additional substance, 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004]. This substance is an α,β-unsaturated aldehyde

and was originally allocated to and evaluated in FGE.224 (EFSA CEF Panel, 2013) in which it was considered not to be of concern with respect to genotoxicity.

In addition, toxicity data have now been provided for the substance 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113]. The data provided are a 14-day (Bauter, 2012) and a 90-day study (Bauter, 2013).

Since the publication of FGE.76, information on European production figures has been provided by EFFA for eight substances, [FL-no: 15.002, 15.005, 15.008, 15.027, 15.029, 15.030, 15.109 and 15.113] (EFFA, 2010; EFFA, 2012; EFFA, 2013a). Furthermore, new information from Industry on missing stereoisomeric composition for [FL-no: 15.022, 15.029, 15.030, 15.032, 15.109 and 15.113] (EFFA, 2013b) and information on solubility in water for [FL-no: 15.005, 15.008, 15.017, 15.018, 15.019 and 15.113]) (EFFA, 2013c) have also been included in the present revision.

2. PRESENTATION OF THE SUBSTANCES IN THE JECFA FLAVOURING GROUP

2.1. Description

2.1.1. JECFA Status

The JECFA has at its 59th meeting in 2002 evaluated a group of 30 flavouring substances consisting of sulphur-containing heterocyclic compounds (JECFA, 2002a; JECFA, 2003).

2.1.2. EFSA Considerations

Two of the 30 sulphur-containing heterocyclic compound are not in the Register [2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture) (JECFA-no: 1046) and 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture) (JECFA-no: 1047)]. One of the substances, 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004], is an α,β-unsaturated aldehyde and one substance, 3-acetyl-2,5-dimethylthiophene [FL-no: 15.024], is an α,β-unsaturated ketone. These two substances were to be evaluated together with other α,β-unsaturated aldehydes and ketones. The α,β-unsaturated aldehyde 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] was evaluated in FGE.224 (EFSA CEF Panel, 2013) in which the substance was considered not to be of concern with respect to genotoxicity. The substance is therefore included in this revision of FGE.76. One substance [FL-no: 15.028] is no longer supported for use as a flavouring substance in Europe and will therefore not be considered any further. This consideration will therefore deals with 26 of the 30 JECFA evaluated substances.

The Panel concluded that all the 26 substances in the JECFA flavouring group of sulphur-containing heterocyclic compounds are structurally related to the group of 59 thiazole, thiophene, thiazoline and thienyl derivatives from chemical group 29 and miscellaneous substances from chemical group 30 evaluated by EFSA in the Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3)[7] (EFSA CEF Panel, 2012). The substances in FGE.21Rev3 were subdivided into a number of subgroups, and the substances in this JECFA evaluated group will be considered in relation to their corresponding EFSA FGE.21Rev3 subgroup.

[7] The Panel is aware that for FGE.21, a revision 4 has been released. For the candidate substances in subgroups B-II and B-III of FGE.21Rev3, a concern with respect to genotoxicity was raised. This concern is also applicable to candidate substances [FL no: 15.029, 15.030 and 15.032] in FGE.76. Since in revision 4 of FGE.21 subgroup B-III has been removed, in order to facilitate the identification of the reason for this concern for these three substances in FGE.76, reference to FGE.21Rev3 is maintained, rather than to FGE.21Rev4.

2.2. Isomers

2.2.1. Status

Six substances [FL-no: 15.022, 15.029, 15.030, 15.032, 15.109 and 15.113] in the group of JECFA evaluated sulphur-containing heterocyclic compounds have one or more chiral centres. Three of these substances can furthermore exist as geometrical isomers [FL-no: 15.029, 15.030 and 15.032].

2.2.2. EFSA Considerations

Adequate information on isomeric composition is available for all six isomeric substances [FL-no: 15.022, 15.029, 15.030, 15.032, 15.109 and 15.113] (Table 1).

2.3. Specifications

2.3.1. Status

The JECFA specifications are available for all 26 substances (JECFA, 2002b). See Table 1.

2.3.2. EFSA Considerations

The available specifications are considered adequate for all 26 substances.

3. INTAKE ESTIMATION

3.1. Status

For all substances evaluated through the JECFA Procedure, intake data are available for the EU (see Table 6).

3.2. EFSA Considerations

For all substances the Industry has submitted production figure for EU and therefore MSDI values for all can be calculated (see Table 6).

SUMMARY OF SPECIFICATION DATA

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Sulphur-Containing Heterocyclic Compounds (JECFA, 2002b)

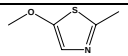
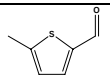
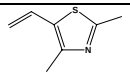
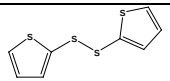
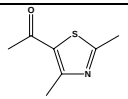
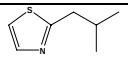
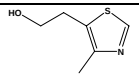
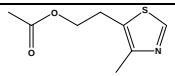
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
15.001 1052	2-Mercaptothiophene		3062 478 7774-74-5	Liquid $C_4H_4S_2$ 116.20	Very slightly soluble Miscible	166 NMR 98 %	1.618-1.622 1.250-1.255	
15.002 1057	2-Methyl-5-methoxythiazole		3192 736 38205-64-0	Liquid C_5H_7ONS 129.18	Insoluble Miscible	117 (44 hPa) MS 98 %	1.515-1.520 1.146-1.154	
15.004 1050	5-Methyl-2-thiophenecarbaldehyde		3209 2203 13679-70-4	Liquid C_6H_6OS 126.18	Practically insoluble or insoluble Miscible	113-114 (33hPa) NMR 95 %	1.574-1.586 1.168-1.172	
15.005 1039	2,4-Dimethyl-5-vinylthiazole		3145 2237 65505-18-2	Liquid C_7H_9NS 139.22	Slightly soluble Miscible	183-184 NMR 99 %	1.560-1.565 1.050-1.056	
15.008 1053	2-Thienyl disulfide		3323 2333 6911-51-9	Solid $C_8H_6S_4$ 230.39	Very soluble Soluble	n.a. 55-60 NMR 98 %	n.a. n.a.	
15.011 1055	5-Acetyl-2,4-dimethylthiazole		3267 2336 38205-60-6	Liquid C_7H_9ONS 155.22	Insoluble Miscible	228-230 NMR 97 %	1.536-1.547 1.147-1.152	
15.013 1034	2-Isobutylthiazole		3134 11618 18640-74-9	Liquid $C_7H_{11}NS$ 141.24	Slightly soluble Miscible	178-180 NMR 96 %	1.490-1.499 0.993-0.997	
15.014 1031	5-(2-Hydroxyethyl)-4-methylthiazole		3204 11621 137-00-8	Liquid C_6H_9ONS 143.21	Soluble Miscible	135 (9 hPa) IR 96 %	1.540-1.556 1.196-1.210	
15.015 1054	4-Methyl-5-(2-acetoxyethyl)thiazole		3205 11620 656-53-1	Liquid $C_8H_{11}O_2NS$ 185.25	Slightly soluble Miscible	117-118 (8 hPa) NMR 97 %	1.505-1.515 1.145-1.149	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Sulphur-Containing Heterocyclic Compounds (JECFA, 2002b)

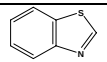
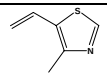
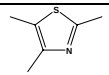
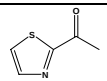
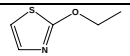
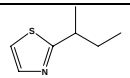
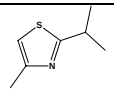
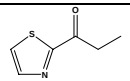
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
15.016 1040	Benzothiazole		3256 11594 95-16-9	Liquid C_7H_5NS 135.19	Very slightly soluble Miscible	231 NMR 96 %	1.637-1.644 1.236-1.240	
15.017 1035	4,5-Dimethylthiazole		3274 11606 3581-91-7	Liquid C_5H_7NS 113.18	Very slightly soluble Miscible	158 (965 hPa) NMR 97 %	1.516-1.524 1.067-1.072	
15.018 1038	4-Methyl-5-vinylthiazole		3313 11633 1759-28-0	Liquid C_6H_7NS 125.19	Very slightly soluble Miscible	78-80 (33 hPa) NMR 97 %	1.560-1.570 1.091-1.095	
15.019 1036	2,4,5-Trimethylthiazole		3325 11650 13623-11-5	Liquid C_6H_9NS 127.21	Very slightly soluble Miscible	65-67 (26 hPa) NMR 97 %	1.503-1.511 1.011-1.015	
15.020 1041	2-Acetylthiazole		3328 11726 24295-03-2	Liquid C_5H_5ONS 127.17	Insoluble Miscible	89-91 (16 hPa) NMR 97 %	1.543-1.550 1.225-1.229	
15.021 1056	2-Ethoxythiazole		3340 11611 15679-19-3	Liquid C_5H_7ONS 129.18	Insoluble Miscible	157-160 NMR 99 %	1.498-1.502 1.131-1.135	
15.022 1033	2-(sec-Butyl)thiazole		3372 11598 18277-27-5	Liquid $C_7H_{11}NS$ 141.24	Slightly soluble Miscible	173-174 NMR 98 %	1.496-1.502 0.998-1.003	Racemate (EFFA, 2013b).
15.026 1037	2-Isopropyl-4-methylthiazole		3555 15679-13-7	Liquid $C_7H_{11}NS$ 141.24	Slightly soluble Miscible	92 (65 hPa) NMR MS 96 %	1.480-1.502 1.001-1.006	
15.027 1042	2-Propionylthiazole		3611 43039-98-1	Liquid C_6H_7ONS 141.19	Insoluble Miscible	95 (1 hPa) IR NMR MS 98 %	1.528-1.533 1.205-1.210	
15.028 1032	Thiazole		3615 11642 288-47-1	Liquid C_3H_3NS 85.13	Slightly soluble Miscible	115-118 IR NMR MS 98 %	1.531-1.541 1.198-1.202	No longer supported by Industry (EFSA, 2011).

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Sulphur-Containing Heterocyclic Compounds (JECFA, 2002b)

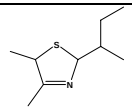
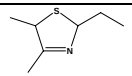
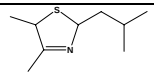
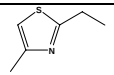
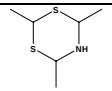
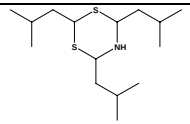
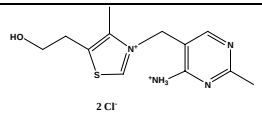
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
15.029 1059	2-(sec-Butyl)-4,5-dimethyl-3-thiazoline		3619 65894-82-8	Liquid $C_9H_{17}NS$ 171.31	Insoluble Miscible	71 (5 hPa) IR NMR MS 98 %	1.483-1.488 0.950-0.955	According to JECFA: Min. assay value is ”98 %” and ”60:40 mix of cis and trans isomers”. Mixture of diastereoisomers, each of them racemic (EFFA, 2013b).
15.030 1058	4,5-Dimethyl-2-ethyl-3-thiazoline		3620 76788-46-0	Liquid $C_7H_{13}NS$ 143.25	Insoluble Miscible	50 (4 hPa) IR NMR MS 98 %	1.490-1.495 1.001-1.010	Mixture of diastereoisomers, each of them racemic (EFFA, 2013b).
15.032 1045	4,5-Dimethyl-2-isobutyl-3-thiazoline		3621 65894-83-9	Liquid $C_9H_{17}NS$ 171.31	Insoluble Miscible	71 (5 hPa) IR NMR MS 97 %	1.483-1.489 0.933-0.937	According to JECFA: Min. assay value is ”97 %” and ”60:40 mix of cis and trans isomers”. Mixture of diastereoisomers, each of them racemic (EFFA, 2013b).
15.033 1044	2-Ethyl 4-methylthiazole		3680 11612 15679-12-6	Liquid C_6H_9NS 127.21	Slightly soluble Miscible	161-162 NMR MS 97 %	1.500-1.510 1.026-1.031	
15.035 1043	4-Methylthiazole		3716 11627 693-95-8	Liquid C_4H_5NS 99.16	Slightly soluble Miscible	133-134 IR NMR MS 97 %	1.519-1.528 1.088-1.092	
15.109 1049	2,4,6-Trimethyldihydro-1,3,5(4*H*)-dithiazine		4018 11649 638-17-5	Solid $C_6H_{13}NS_2$ 163.30	Insoluble Miscible	n.a. 48 IR NMR MS 99 %	n.a. n.a.	Mixture of diastereoisomers, each of them racemic (EFFA, 2013b).

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Sulphur-Containing Heterocyclic Compounds (JECFA, 2002b)

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
15.113 1048	5,6-Dihydro-2,4,6,tris(2-methylpropyl)-4*H*-1,3,5-dithiazine		4017 74595-94-1	Solid $C_{15}H_{31}NS_2$ 289.55	Slightly soluble Miscible	n.a. 33-35 IR NMR 95 %	n.a. n.a.	According to JECFA: Min. assay value is ”95 % (mixture of 3 stereoisomers).” Mixture of diastereoisomers, each of them racemic (EFFA, 2013b).
16.027 1030	Thiamine hydrochloride		3322 10493 67-03-8	Solid $C_{12}H_{18}ON_4S$ 337.27	Soluble Slightly soluble	n.a. 248-250 NMR 98 %	n.a. n.a.	

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.
n.a. Not applicable.

4. GENOTOXICITY DATA

4.1. Genotoxicity Studies – Text Taken[8] from the JECFA Report (JECFA, 2003)

In Vitro

Three substances, thiazole [FL-no: 15.028], 4,5-dimethylthiazole [FL-no: 15.017] and 4-methylthiazole (FL-no: 15.035), in this group of flavouring substances were tested for their ability to induce reverse mutation in *Salmonella typhimurium* strains TA98 and TA100 at doses of 4 - 100 µmol/plate. The purity of the chemicals was not stated. Positive results were obtained with thiazole in strain TA100 only at a minimum concentration of 4 µmol/plate; however, the mutagenicity that was observed in the absence of an exogenous metabolic activation system was less marked in the presence of such a system, indicating that thiazole does not undergo metabolic activation. The other substances gave uniformly negative results in both strains (Lee et al., 1994). No other tests have been reported.

For a summary of *in vitro* genotoxicity data considered by the JECFA, see Table 2.

4.2. Genotoxicity Studies – Text Taken[9] from EFSA FGE.21Rev3 (EFSA CEF Panel, 2012)

In Vitro

Genotoxicity data were provided for 12 of the candidate substances. These 12 substances belong to subgroup A-Ia: thiophene [FL-no: 15.106]; subgroup A-Ib: 2-methylthiophene [FL-no: 15.091], 3-methylthiophene [FL-no: 15.092], 2,5-dimethylthiophene [FL-no: 15.064], 2-acetylthiophene [FL-no: 15.040], 2-acetyl-3-methylthiophene [FL-no: 15.037], thiophene-2-carbaldehyde [FL-no: 15.107], 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074]; subgroup A-II: 2,4-dimethylthiazole [FL-no: 15.062]; subgroup A-III: 2-methyl-4,5-benzothiazole [FL-no: 15.088]; subgroup B-III: 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099]. There were also mutagenicity data on four supporting substances and on four other structurally related substances. All available information on genotoxicity of the 12 candidate and the four supporting substances and of four other structurally related substances is based upon *in vitro* studies only.

Subgroup A-I:

Thiophene [FL-no: 15.106], 2-methylthiophene [FL-no: 15.091], 3-methylthiophene [FL-no: 15.092] and 2,5-dimethylthiophene [FL-no: 15.064] were reported to be negative in microbial mutagenicity assays. 2-Acetylthiophene [FL-no: 15.040] was negative in microbial tests, using *Salmonella typhimurium* strains TA98 and TA100, with and without metabolic activation and in the SOS chromotest with metabolic activation. 2-Acetylthiophene was reported to be positive without metabolic activation in the SOS *Escherichia coli* chromotest (Mosier et al., 2003). In the same study, 2-acetyl-3-methylthiophene [FL-no: 15.037], thiophene-2-carbaldehyde [FL-no: 15.107] and 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] gave positive results without metabolic activation in the SOS *E. coli* chromotest. The concentrations tested were not reported for any of the substances subjected to the SOS *E. coli* chromotest (Mosier et al., 2003). The Panel considered the endpoint of this test inappropriate for the estimation of genotoxic potential. The supporting substance 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] was negative in a microbial mutagenicity assay.

Thiophene was tested in accordance with OECD guidelines in a bacterial reverse mutation test in strains of *S. typhimurium* and in strain WP2 uvrA of *E. coli*. No evidence of mutagenic response was reported when strains TA100, TA1535, TA98 and TA1537 of *S. typhimurium* were incubated at concentrations of 0, 78.1, 156, 313, 625, 1250, 2500 and 5000 µg/plate with and without S9 metabolic activation. Toxicity was observed at 1250 µg/plate in TA1537, and 2500 µg/plate in strains TA100,

[8] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

[9] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

TA1535 and TA98 also with and without metabolic activation. Toxicity was observed at 5000 µg/plate in WP2 with and without S9 metabolic activation (Shibuya, 2006).

In a chromosomal aberration test, thiophene was tested on Chinese hamster lung cells in accordance with Japanese Guidelines. No chromosomal aberrations or polyploidy was reported when incubated with concentrations of 0, 210, 420, 840 µg/mL of thiophene, with and without metabolic activity (Tanaka, 2006).

Subgroup A-II:

2,4-Dimethylthiazole [FL-no: 15.062] was reported to be negative in microbial assays, using *S. typhimurium,* but only in strain TA100 and only in the absence of metabolic activation (Voogd et al., 1983). Two supporting substances, 4,5-dimethylthiazole [FL-no: 15.017] and 4-methylthiazole [FL-no: 15.035], were negative in microbial mutagenicity assays.

Subgroup A-III:

2-Methyl-4,5-benzothiazole [FL-no: 15.088] was reported to be negative in an Ames test but only a summary report was available (Longfellow, 1998). The supporting substance benzothiazole [FL-no: 15.016] was negative in microbial mutagenicity assay and in the mouse lymphoma test.

Subgroups B-I and B-II:

No genotoxicity information was available for any candidate or supporting substances in these subgroups. However, considering the structural similarities between the thiazolines in subgroup B-II and the thiazolidines in subgroup B-III, the Panel also concluded that the thiazolines [FL-no: 15.060, 15.086 and 15.119] could not be evaluated through the Procedure (see Subgroup B-III below).

Subgroup B-III:

The two candidate substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] as well as the structurally related ethyl, isopropyl, *n*-butyl and isobutyl thiazolidine have all been reported to be positive in the Ames tests (TA98 and TA100) (Mihara and Shibamoto, 1980). Owing to limited reporting, the data could not be properly evaluated. Nevertheless, these reports do raise the possibility of a genotoxic potential of these thiazolidines. Accordingly, it was concluded not to evaluate the candidate substances 2-methylthiazolidine and 2-propylthiazolidine through the Procedure.

Subgroup B-IV:

No genotoxicity information was available for any candidate or supporting substance in this subgroup.

Subgroup B-V:

The two candidate substances 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133] are α,β-unsaturated ketones i.e. they have a structural alert for genotoxicity (EFSA, 2008) and as there are no genotoxicity data available a concern for genotoxicity cannot be ruled out.

Subgroup B-VI:

No genotoxicity information was available for any candidate or supporting substance in this subgroup.

Conclusion on genotoxicity

It is concluded that the genotoxicity data are limited and that genotoxicity could not be assessed adequately for the flavouring substances in the present revision of FGE.21, Revision 3. However, except for the two dihydrothiazines, 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133], the two thiazolidines 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] and the three structurally related thiazolines 2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], the genotoxicity data available do not preclude the evaluation of the remaining 49 candidate substances using the Procedure.

For a summary of *in vitro* genotoxicity data considered by EFSA, see Table 3.

4.3. Genotoxicity Studies and Conclusion on Genotoxicity and Carcinogenicity - Text Taken[10] from FGE.224 (EFSA CEF Panel, 2013)

The Industry has submitted data concerning genotoxicity studies for one substance, 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004]. The new data submitted covers both *in vitro* and *in vivo* genotoxicity assays.

In vitro data on 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004]

Bacterial Reverse Mutation Assay

5-Methyl-2-thiophenecarbaldehyde was tested for the induction of gene mutations in the *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 both in the absence and in the presence of Aroclor induced rat liver S9-mix. Three independent experiments were performed (Beevers, 2009). An initial toxicity range-finding experiment was carried out in the absence and presence of S9-mix in strain TA100. Six concentrations were tested in the concentration range 1.6 - 5000 µg/plate of 5-methyl-2-thiophenecarbaldehyde. Negative (solvent) and positive controls were included. Toxicity, evident as a decrease in revertant count, was apparent on all plates treated at 1000 µg/plate and above in the absence and presence of S9-mix, but revertant counts were obtained from at least four different concentrations, and these data were included as part of experiment 1.

In the first main experiment, 5-methyl-2-thiophenecarbaldehyde was tested in the remaining 4 strains in the absence and presence of S9-mix using the plate incorporation methodology at concentrations ranging from 0.32 - 1000 µg/plate. Based on the range finding study the maximum tested concentration was reduced to 1000 µg/plate. Evidence of toxicity was observed at 200 µg/plate and above in strains TA1537 and TA102, in the presence of S9-mix, and at 1000 µg/plate in strains TA98 and TA102 in the absence of S9-mix and in strains TA98 and TA1535 in the presence of S9-mix. However, revertant counts were obtained from six different concentrations, and so the data were considered valid for evaluation.

In a second experiment, treatments of all the tester strains were performed in the absence and presence of S9-mix. For each strain the highest tested concentration was based on toxicity in the first experiment and narrowed concentration ranges were employed. In addition, all treatments in the presence of S9-mix were further modified by the inclusion of a 1-hour pre-incubation step. Clear evidence of toxicity was observed in strains TA98 and TA102 following treatment at the maximum test concentration in both the absence and presence of S9-mix, and in the strain TA1537 following treatment at the maximum concentration in the presence of S9-mix. However, toxicity was not seen at the concentrations tested in TA100 and TA1535 in the presence and absence of S9-mix or in TA1537 in the absence of S9-mix, and therefore it was considered that higher concentrations should be evaluated. For the other strains data from a sufficient number of concentrations were obtained.

[10] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

In the third experiment, 5-methyl-2-thiophenecarbaldehyde was tested in TA100 and TA1535 in the absence and presence of S9-mix and in TA1537 in the absence of S9-mix at 156.25 - 5000 µg/plate. Following these treatments, evidence of toxicity was observed at 2500 µg/plate and above in strains TA100 and TA1535 in the presence of S9-mix only.

No statistically significant increases in revertant numbers were observed in any of the tester strains that were both concentration-related and clearly reproducible. Some small increases in revertant numbers were observed in strain TA1535 in the absence of S9-mix, but these were sporadic, not concentration related and not reproducible. They were therefore considered to be chance occurrences and not a compound-related effect and therefore not biological relevant.

It was concluded that 5-methyl-2-thiophenecarbaldehyde did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *S. typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to either the limit of toxicity or 5000 µg/plate (the maximum recommended concentration according to current regulatory guidelines), in both the absence and in the presence of a rat liver metabolic activation system (S9-mix).

For validation and study results, see Table 4.

In Vitro Micronucleus assays

5-Methyl-2-thiophenecarbaldehyde was tested for the induction of chromosome damage and potential aneugenic effects in an *in vitro* micronucleus assay using duplicate human peripheral blood lymphocytes prepared from pooled blood from two healthy volunteers in two separate experiments. Treatments were performed both in the absence and presence of Aroclor 1254 induced rat liver S9-mix (Lloyd, 2011). Experiment 1 was conducted using blood from female donors and Experiment 2 was conducted using blood from male donors.

Treatment with 5-methyl-2-thiophenecarbaldehyde was conducted 48 hours following culture initiation (stimulation by phytohaemagglutinin).

A preliminary toxicity range-finding experiment was conducted with S9-mix and 3 hours treatment and without S9-mix with 3 and 24 hours treatment. Toxicity was evaluated as the effect of treatment on the Replication Index (RI). Twelve concentrations from 4.6 to 1262 µg/mL were tested. The concentrations selected for the main experiments were based on toxicity data from this preliminary test.

In experiment 1 (female donors), cells were exposed to 5-methyl-2-thiophenecarbaldehyde for 3 hours and 21 hours recovery (21 + 3) both with and without S9-mix. In addition, a continuous 24 hours treatment without recovery (24 + 0) was performed without S9 mix. All cultures were sampled 24 hours after the beginning of treatment (i.e. 72 hours after culture initiation). The concentrations selected for evaluation in the absence of S9-mix and 3 hours exposure were 600, 900 and 1000 µg/mL and in the presence of S9-mix and 3 hours exposure 50, 60 and 70 µg/mL. After 24 hours exposure, cultures exposed to 120 µg/mL, 240 µg/mL, 300 µg/mL and 350 µg/mL were evaluated. Relevant positive and negative controls were included in all experiments. At the first test conditions (3 + 21 hours without S9-mix) no significant increases in the frequency of micronucleated binucleate cells (MNBN) were observed relative to concurrent vehicle controls at all concentrations analysed. Furthermore, the MNBN cell frequencies in all treated cultures under this treatment condition fell within the 95th percentile of the normal range.

In the 3 + 21 hours treatment condition with S9-mix the frequency of MNBN cells were significantly higher (1.05 %, 1.03 % and 1.33 % at 50, 60 and 70 µg/mL, respectively) ($p \leq 0.001$) than concurrent controls (0.31 %) at all concentrations analysed. The initial analysis of 1000 binucleate cells/culture revealed increased MNBN cell frequencies that exceeded the 95th percentile of the normal range for

female donors in one of the two replicate cultures at 50 and 60 µg/mL and in both replicate cultures at 70 µg/mL. Following the additional analysis of 1000 binucleate cells/culture from the vehicle controls and the test concentrations, the frequencies of MNBN cells were still significantly higher ($p \leq 0.001$) than those observed in concurrent controls at all three concentrations analysed. The MNBN cell frequencies in one replicate culture at 60 µg/mL and in both replicate cultures at 70 µg/mL (1.33 %) exceeded the 95th percentile of the normal range (0.1 - 1.2 %), however, both cultures at 50 µg/mL (1.05 %) fell within the normal range. These observations are indicative of a weak induction of micronuclei.

As a follow up of this positive result a second experiment was performed with lymphocytes from male donors to explore whether the weak induction of micronuclei that was observed in Experiment 1 in the presence of S9-mix could be due to the low MN frequencies in control cultures from female blood donors. Following treatment for 3 hours in the presence of S9-mix with 5-methyl-2-thiophenecarbaldehyde at concentrations of 50 µg/mL, 60 µg/mL, 70 µg/mL and 80 µg/mL, followed by 21 hours recovery, and analysis of 1000 binucleate cells/culture, the frequencies of MNBN cells (0.9 %) were significantly higher ($p \leq 0.001$) at 70 µg/mL compared to concurrent vehicle controls (0.30 %). The MNBN cell frequencies in single replicate cultures at 70 and 80 µg/mL exceeded the 95th percentile of the normal range for male donors (0.0 - 0.7 %) but the MNBN frequency at 80 µg/mL fell within the normal range. There was a concentration-dependent MN response from 50 - 70 µg/mL, with 70 µg/mL exceeding the normal range (0.90 %). An additional 1000 binucleate cells/culture were analysed, and as a result of the additional scoring the MNBN cell frequencies were significantly higher ($p \leq 0.05$) than concurrent vehicle controls at the three highest concentrations analysed (60, 70 and 80 µg/mL). However, the cumulative MNBN cell frequencies exceeded the normal range at only the 70.00 µg/mL concentration (and attributable to only one of two cultures). These results are again indicative of weak induction of micronuclei.

In all of the different treatment conditions and separate experiments, negative control frequencies of MNBN were normal and were significantly increased by treatment with the positive control chemical.

In conclusion, 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] weakly induced micronuclei in both male and female human peripheral blood lymphocytes cultures when tested for 3 + 21 hours in the presence of S9-mix. In the same test system 5-methyl-2-thiophenecarbaldehyde did not induce micronuclei at up to toxic concentrations for 3 + 21 hours and 24 + 0 hours in the absence of S9-mix.

For validation and study results, see Table 4.

In Vivo data on 5-Methyl-2-thiophenecarbaldehyde [FL-no: 15.004]

In Vivo Combination Assay (Comet + Micronucleus)

On the basis of the *in vitro* micronucleus study reported above, as a next step to probe the genotoxic potential of 5-methyl-2-thiophenecarbaldehyde, a combined Comet assay and an *in vivo* micronucleus assay was carried out in rats (Beevers, 2012). This combined approach minimised the number of animals used in the experiments. Micronuclei were measured in bone marrow, but additionally, the liver was chosen as the most appropriate tissue for analysis in the Comet assay due to the fact that S9 metabolic activation was necessary to produce weakly positive results in the *in vitro* micronucleus assay, and this organ is the primary site of metabolism. Therefore, groups of Han Wistar male rats were administered 5-methyl-2-thiophenecarbaldehyde via gavage and the liver and bone marrow were analysed for the potential induction of DNA damage.

An initial dose range finding study was conducted to estimate the Maximum Tolerated Dose (MTD) of 5-methyl-2-thiophenecarbaldehyde after administration by oral gavage to groups of three male and three female Han Wistar rats. Doses of 1000 mg/kg bw/day resulted in mortality in both male and female rats while at 700 mg/kg bw/day mortality occurred in the female group but not in the male group. On this basis, 700 mg/kg bw/day was considered the MTD in males and 500 mg/kg bw/day was

considered the MTD in females. Although there was a slight difference in MTD between males and females, it was less than 2-fold. Moreover, below 700 mg/kg bw/day no gender differences in clinical signs of toxicity were observed. It was therefore concluded that male rats alone could be used in the combined Comet and micronucleus assay.

Groups of six male Han Wistar rats were treated by oral gavage with 5-methyl-2-thiophenecarbaldehyde at doses of 70, 350 and 700 mg/kg bw/day, including a vehicle control (5 % w/v aqueous methylcellulose) and a positive control (ethyl methanesulphonate, 150 mg/kg bw/day). Animals were dosed at 0, 24 and 45 hours. Clinical signs of toxicity and body weight were recorded at each time point within the study. Three hours after the last dose (i.e. at 48 hours) the liver and one femur were removed from each control (negative and positive) and each treated animal for analysis of comets and micronuclei respectively. In a satellite group of animals (N = 3 per group) dosed similarly, 0.5 mL samples of blood were taken from the jugular vein at 0.5, 1, 2, 4, 8 and 24 hours after the final dose in case bioanalytical proof of exposure was subsequently needed.

No clinical signs of toxicity were observed for any animal in the treatment or control groups. No effect of treatment on body weight was observed. Clinical chemistry results did not present marked changes between treatment or control groups with two exceptions. Levels of aspartate aminotransferase were increased following dosing at 700 mg/kg bw/day compared to control values. Additionally, a histological observation of glycogen deposits in the liver of animals dosed at 350 and 700 mg/kg bw/day, along with changes in liver enzymes, indicate that the liver was exposed to the test article, 5-methyl-2-thiophenecarbaldehyde. These observations indicated exposure to the target organ (the liver) of the Comet assay (see below).

In the micronucleus assay femoral bone marrow was filtered through cellulose columns to remove the majority of nucleated cells, smears were made, fixed and stained with acridine orange. Two thousand polychromatic erythrocytes (PCE) per animal were scored for micronuclei under fluorescence microscopy. The data revealed that rats treated with 5-methyl-2-thiophenecarbaldehyde at all doses exhibited group mean % PCE (out of total erythrocytes) that were similar to the vehicle control group confirming there was no evidence of test article-related bone marrow toxicity. Micronucleus frequencies in vehicle control rats were normal and were significantly increased by positive control treatment. Rats treated with 5-methyl-2-thiophenecarbaldehyde at all doses exhibited micronuclei PCE frequencies that were similar to the vehicle control group and which were considered consistent with the laboratory's historical data. There were no statistically significant increases in micronucleus frequency for any of the groups receiving the test article, compared to the concurrent vehicle control. There was no evidence of bone marrow toxicity, and therefore no direct evidence that the substance did reach the bone marrow. Therefore, no firm conclusion could be drawn on this part of the study.

In the alkaline Comet assay, liver samples from all control and test article treated animals were washed thoroughly, cut into small pieces in Merchants solution and then pushed through bolting cloth to produce single cell suspensions. Four slides were prepared per single cell suspension. Single cells were imbedded in agarose and once gelled, all slides were placed overnight in lysis buffer. Following lysis 3 of the 4 slides for each tissue and animal were transferred to electrophoresis buffer (pH >13) and the DNA unwound for 30 minutes and were electrophoresed in the same buffer at 0.7 V/cm for 40 minutes. After the lysis step, the 4th slide from each tissue and animal was placed in pH 7.0 buffer for approximately 3 x 5 minutes and then dried. This ‘diffusion’ slide was used to estimate the degree of damaged cells in the cell suspensions.

After staining with ethidium bromide tail moment and tail intensity (% DNA in tail) were obtained from 100 cells/animal/tissue (50 cells from each of two slides, where possible). Each slide was examined for possible indications of cytotoxicity. The number of 'clouds' out of 100 cells was scored for each slide. 'Clouds' were not used for comet analysis. Vehicle control animals exhibited quite low comet scores, but significant DNA damage was induced by the positive control. The Comet analysis revealed that animals treated with 5-methyl-2-thiophenecarbaldehyde exhibited elevated mean tail

intensities and tail moments compared to concurrent vehicle control animals. However, the majority of animals, including the vehicle controls, had tail intensity values below the laboratory's historical control range and the elevated mean level was due to only one animal in each group. Thus, the data generated for this assay is considered to fall within the normal level of variation for the assay. In addition, there was no indication of dose response relationship. Therefore it is considered that 5-methyl-2-thiophenecarbaldehyde does not induce DNA damage in the livers of rats when administered by oral gavage up to the MTD of 700 mg/kg bw/day.

Although the Panel noted that the negative control values were extremely low (mean tail intensity of 0.07) the assay was found acceptable because the positive control (EMS) was clearly positive (mean tail intensity of 29.43). The main problem with a low negative control value is that a test with low negative control values may have a difficulty to identify DNA crosslinking substances (with two reactive groups). However, the chemical structure of the test substance does not indicate a crosslinking potential. No studies on metabolism of 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] are available to the Panel. The CEF Panel recently (EFSA CEF Panel, 2011) evaluated a structural related substance, 5-methyl furfural in FGE.66Rev1 (EFSA CEF Panel, 2011) to have no concern for genotoxicity. The most likely metabolic conversions of 5-methyl furfural [FL-no: 13.001] are oxidation of the aldehyde group to the carboxylic acid followed by conjugation with e.g. glycine or glucuronide, with rapid elimination in the urine. For furan and alkylfurans, ring opening has also been described, which would result in the formation of highly reactive unsaturated dialdehydes. In order to give an indication of whether ring opening could be possible for 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] an "evaluation" of the metabolism of this substance was run in a prediction programme (METEOR NEXUS version 1.5). In this programme no indications of ring opening were generated. Overall the Panel considered that the formation of a bifunctional DNA reactive metabolite is unlikely, and therefore concludes that this substance is not likely to have a cross-linking potential. The negative result of the Comet assay in the liver is considered acceptable.

For validation and study results, see Table 5.

Conclusion on genotoxicity for 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004]

5-Methyl-2-thiophenecarbaldehyde [FL-no: 15.004] did not induce mutations in a gene mutation test in bacteria (Ames test). It did, however, induce weak genotoxic effects in an *in vitro* micronucleus assay in the presence of S9-mix. However, these weakly positive *in vitro* results were not confirmed in an *in vivo* combination assay (Comet assay in liver + micronucleus in bone marrow) in male rats when dosed up to the MTD. The Panel therefore concluded that 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] does not give rise to concern with respect to genotoxicity and can accordingly be evaluated using the Procedure.

For validation and study results, see Table 4 and 5.

4.4. EFSA Considerations

The Panel concluded that the three 3-thiazolines, 2-(sec-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029], 4,5-dimethyl-2-ethyl-3-thiazoline [FL-no: 15.030] and 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] are structurally related to 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099], evaluated by the Panel in FGE.21Rev3 and reported to be positive in the Ames test (TA98 and TA100). In parallel with its conclusion on the subgroup B-II (thiazolines) in FGE.21Rev3, the Panel concluded that the Procedure could not be applied to these three thiazolines, [FL-no: 15,029, 15.030 and 15.032] until adequate *in vivo* genotoxicity data become available. Additionally, the Panel noted the presence of a terminal conjugated double bond in the substances 2,4-dimethyl-5-vinylthiazole [FL-no: 15.005] and 4-methyl-5-vinylthiazole [FL-no: 15.018], which raised concern for genotoxicity. The Panel decided that the Procedure should not be applied to these two substances either until genotoxicity data become available due to the possibility of formation of reactive metabolites via epoxidation.

Based on the new *in vitro* and *in vivo* genotoxicity studies on 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004], the Panel concluded in FGE.224 (EFSA CEF Panel, 2013) that 5-methyl-2-thiophenecarbaldehyde does not give rise to concern with respect to genotoxicity and can accordingly be evaluated using the Procedure.

The Panel concluded that the data available do not preclude evaluation of the remaining 21 JECFA evaluated sulphur-containing heterocyclic compounds through the Procedure.

5. 14-DAY AND 90-DAY STUDY ON 5,6-DIHYDRO-2,4,6-TRIS(2-METHYLPROPYL)-4*H*-1,3,5-DITHIAZINE [FL-NO: 15.113]

A 14-day range-finding dietary study was performed with 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113] (Bauter, 2012). The study was performed according to OECD Guideline (TG 407). Groups (3/sex/dietary intake level) of male and female Hsd:SD® rats were fed a diet containing 0 (dietary control), 120, 1200 and 2400 mg 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine per kg diet. These dietary levels were equivalent to daily intakes of 0, 11.3, 111.2 and 217.0 mg/kg bw for males and 0, 11.2, 107.2 and 206.1 mg/kg bw for females, respectively. Clinical observations were recorded daily and body weights and food consumption observations were made on day 0, 7 and 14. No mortality was observed throughout the course of the study and the general condition of the rats was unremarkable. No gross pathology was related to the test-substance.

A 90-day dietary study was performed with 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113] (Bauter, 2013). The study was performed according to OECD Guideline (TG 408). Four groups of rats (10/sex/dietary intake level) of male and female CRL Sprague-Dawley CD®IGS rats were fed a diet containing 0 (dietary control) 140, 1050 and 2100 mg of 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine per kg diet. These dietary levels were equivalent to daily intakes of 0, 9.3, 67.9 and 131.9 mg/kg bw for males and 0, 11.0, 77.1 and 153.7 mg/kg bw for females, respectively (Bauter, 2013). The animals were observed daily for signs of gross toxicity, viability and behavioural changes. Clinical observations of toxicity were performed on day 0 and weekly until sacrifice. Animals were weighed on day 0 at the start of the study and weekly thereafter. Food consumption and efficiency were measured and calculated weekly. Blood chemistry and haematology were performed on blood drawn via sublingual bleed during week 12 after overnight fast and coagulation assessment was performed prior to necropsy. Urine was collected during the 15 hours prior to the blood draw. Prior to initiation of the study and on day 91 the eyes of all rats were examined by focal illumination and indirect ophthalmoscopy. At termination of the study all animals were sacrificed and subject to full necropsy. The following tissues were weighed wet post dissection: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, testes, spleen, thymus, uterus with oviducts. Histopathology was performed on a comprehensive number of tissues and organs in accordance with the guidelines.

No mortality was observed in any group throughout the study. There were no toxicologically significant or dose-related differences in animals between treatment and control groups in food consumption or food efficiency, body weight and body weight gain, and clinical or ophthalmological parameters. Only a reduction in food consumption in females at the highest dose was reported to be statistically significant, which, however, was not accompanied with body weight changes and was not considered adverse or biologically relevant. No treatment-related differences in clinical or gross pathology or in organ weights were observed. Treatment-related microscopic findings were reported in the urinary bladder of males and females of the two highest dose groups (1050 and 2100 mg/kg diet) and involved statistically significant increase in the incidence of minimal to slight simple and diffuse hyperplasia of the mucosal epithelium with increased severity in the highest dose group. This finding was not correlated to any other clinical or pathological changes.

The author concluded that under the conditions of the study, the no-observed-adverse-effect level (NOAEL) for dietary administration of 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine was determined to be 140 mg/kg diet, equivalent to an estimated daily intake of 9.3 mg/kg bw/day for males and 11.0 mg/kg bw/day for females, respectively, based on the effects noted on the urinary bladder. The Panel agrees to this conclusion and used 9.3 mg kg bw/day in the margin of safety assessment.

6. APPLICATION OF THE PROCEDURE

6.1. Application of the Procedure to 26 Sulphur-Containing Heterocyclic Compounds by the JECFA (JECFA, 2002a)

According to the JECFA 17 of the substances belong to structural class II and nine to structural class III using the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

The JECFA concluded on three sulphur-containing heterocyclic compounds [FL-no: 15.014, 15.015 and 16.027] at step A3 in the JECFA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and that the intakes for two of the substances [FL-no: 15.014 and 15.015] are below the thresholds for their structural class II (step A3). For one substance, thiamine hydrochloride [FL no: 16.027], the intake was above the threshold for structural class II and the substance is considered not to occur endogenously in humans, therefore the evaluation proceeded to step A5, where it was considered as of no safety concern at the estimated level of intake based on a 90-day dietary study in rats in which a No Observed Adverse Effect Level (NOAEL) of 36 mg/kg body weight (bw)/day provides a margin of safety of more than 500.

Twenty-three substances were concluded at step B4 in the JECFA Procedure, i.e. the substances are not expected to be metabolised to innocuous products and the estimated intakes are below the thresholds for their structural classes II and III. An adequate NOAEL was available for all 23 substances and the JECFA concluded that the substances are therefore not expected to be of safety concern when used as flavouring substances.

In conclusion, the JECFA evaluated all 26 substances to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the 26 sulphur-containing heterocyclic compounds are summarised in Table 6: Summary of Safety Evaluation of Sulphur-Containing Heterocyclic Compounds (JECFA, 2003).

6.2. Application of the Procedure to 59 Thiazoles, Thiophene, Thiazoline and Thienyl Derivatives and Miscellaneous Substances from Chemical Group 30 by EFSA (FGE.21Rev3) (EFSA CEF Panel, 2012)

Fifty-nine candidate substances were evaluated in FGE.21Rev3. Fourty-eight substances are classified into structural class II and 11 into structural class III using the decision tree approach presented by (Cramer et al., 1978).

For seven substances the Procedure could not be applied due to indication of genotoxic potential *in vitro* [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133].

The substances were allocated into structural subgroups (for description and explanation, see FGE.21Rev3) and were evaluated at step B4 in the Procedure, i.e. the substances are not expected to be metabolised to innocuous products and the estimated intakes are below the thresholds for their structural classes II and III.

In summary, the Panel concluded that 26 of the candidate substances evaluated through the Procedure, from the structural subgroups A-Ic (thiophenes with thiol-containing ring substituents) and A-II (thiazoles) are not of safety concern at their estimated levels of intake based on the MSDI approach,

whereas for 26 candidate substances from the structural subgroups A-Ia (thiophene), A-Ib (thiophenes with non-thiol-containing ring substituents), A-III (benzothiazoles), B-I (dihydrothiophenes), B-IV (dithiazines) and B-V (dihydrothiazines) additional data are required.

The stepwise evaluations of the 59 substances are summarised in Table 7: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.21Rev3) (EFSA CEF Panel, 2012).

6.3. EFSA Considerations

The Panel agrees with the application of the Procedure, as performed by the JECFA, for 21 of the 26 substances in the group of sulphur-containing heterocyclic compounds. Three of the 26 substances evaluated by the JECFA, 2-(sec-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029], 4,5-dimethyl-2-ethyl-3-thiazoline [FL-no: 15.030] and 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] were considered by the Panel to have genotoxic potential *in vitro*, and therefore the Panel concluded that the Procedure should not be applied to these three flavouring substances until adequate *in vivo* genotoxicity data become available. Additionally, the Panel noted the presence of a terminal conjugated double bond in the substances 2,4-dimethyl-5-vinylthiazole [FL-no: 15.005] and 4-methyl-5-vinylthiazole [FL-no: 15.018] which raised concern for genotoxicity. The Panel concluded, contrary to the JECFA, that the Procedure should not be applied to these two substances either until genotoxicity data become available.

For the three substances [FL-no: 15.014, 15.015 and 16.027], expected to be metabolised to innocuous products (A-side), the Panel agrees with the JECFA evaluation.

For 18 of the remaining 21 substances the Panel agreed with the JECFA that they can not be expected to be metabolised to innocuous products. The 18 substances were allocated to one of the 10 structural subgroups identified in FGE.21Rev3 (for description and explanation, see FGE.21Rev3). Taking these substances through the Procedure, it can be estimated that the intakes (MSDI) are below the thresholds for their structural classes II and III, and as the JECFA concluded that adequate NOAELs provides a sufficient safety margin, these substances were concluded at step B4 in the Procedure to be of no safety concern by the JECFA.

For 16 of these 18 substances, from the structural subgroups: A-Ib (thiophenes with non-thiol-containing ring substituents [FL-no: 5.004]), A-Ic (thiophenes with thiol-containing ring substituents [FL-no: 15.001 and 15.008]), A-II (thiazoles [FL-no: 15.002, 15.011, 15.013, 15.017, 15.019, 15.020, 15.021, 15.022, 15.026, 15.027, 15.033 and 15.035]) and A-III (benzothiazoles FL-no: 15.016]), the Panel agrees with the JECFA conclusion that these substances are not expected to be of safety concern when used as flavouring substances, as summarised in Table 6.

For the remaining two of the 18 substances, 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113] and 2,4,6-trimethyldihydro-1,3,5(4*H*)-dithiazine [FL-no: 15.109] both from the structural subgroup B-IV (dithiazines), the intakes (MSDI) of 2.4 and 1.1 µg/capita/day, respectively, are below the threshold for their structural class II. The Panel considered that an adequate NOAEL of 9.3 mg/kg bw/day for [FL-no: 15.113] could be obtained from the new 90-day rat study, which at step B4 in the Procedure provides a sufficient safety margin of 2.3×10^5 for [FL-no: 15.113]. This NOAEL of 9.3 mg/kg bw/day for [FL-no: 15.113] can be used to support the structurally related 2,4,6-trimethyldihydro-1,3,5(4*H*)-dithiazine [FL-no: 15.109], providing a safety margin of 5.1×10^5 for [FL-no: 15.109]. So, it is concluded that these two flavouring substances, 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113] and 2,4,6-trimethyldihydro-1,3,5(4*H*)-dithiazine [FL-no: 15.109], were neither of safety concern, based on the MSDI approach.

CONCLUSION

In Flavouring Group Evaluation 76 (FGE.76), the EFSA considered 26 sulphur-containing heterocyclic compounds evaluated by the JECFA at its 59th meeting. Since publication of FGE.76 one substance, thiazole [FL-no: 15.028], is no longer supported by Industry for use as a flavouring

substance in Europe and will therefore not be considered any further. The present revision is made due to inclusion of one additional substance, 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004], cleared for genotoxicity concern in FGE.224. Additionally, new toxicity data have become available for 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113]. Therefore, the present revision of FGE.76, FGE.76Rev1, considers 26 flavouring substances evaluated by the JECFA.

In Flavouring Group Evaluation 76 (FGE.76), the EFSA considered 26 sulphur-containing heterocyclic compounds evaluated by the JECFA at its 59th meeting. Since publication of FGE.76, one substance, thiazole [FL-no: 15.028], is no longer supported by Industry for use as a flavouring substance in Europe and will therefore not be considered any further. The present revision is made due to inclusion of one additional substance, 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004], cleared for genotoxicity concern in FGE.224. Additionally, new toxicity data have become available for 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113]. Therefore, the present revision of FGE.76, FGE.76Rev1, considers 26 flavouring substances evaluated by the JECFA.

The Panel concluded that all 26 Register substances in the JECFA flavouring group of sulphur-containing heterocyclic compounds are structurally related to the 59 thiazoles, thiophene, thiazoline and thienyl derivatives from chemical group 29 and miscellaneous substances from chemical group 30 evaluated by EFSA in the Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3).

In the previous version of FGE.76, the Panel considered that for the substances [FL-no: 15.109 and 15.113] no adequate NOAELs were available. Since then a 90-day study has become available for 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113] providing a NOAEL to establish adequate margins of safety for the substance as well as for the structurally related 2,4,6-trimethyldihydro-1,3,5(4*H*)-dithiazine [FL-no: 15.109].

The Panel agrees with the application of the Procedure as performed by the JECFA for 21 of the 26 substances considered in this FGE. Three of the remaining five substances, 2-(sec-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029], 4,5-dimethyl-2-ethyl-3-thiazoline [FL-no: 15.030] and 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] were considered by the Panel to have genotoxic potential *in vitro*, and therefore the Panel decided that the Procedure should not be applied to these three flavouring substances until adequate *in vivo* genotoxicity data become available. Additionally, the Panel noted the presence of a terminal conjugated double bond in the substances 2,4-dimethyl-5-vinylthiazole [FL-no: 15.005] and 4-methyl-5-vinylthiazole [FL-no: 15.018] which raised concern for genotoxicity. The Panel concluded that the Procedure should not be applied to these two substances either until additional data become available.

Thus, the Panel agreed that the Procedure can be applied to 21 of the 26 JECFA-evaluated substances [FL-no: 15.001, 15.002, 15.004, 15.008, 15.011, 15.013, 15.014, 15.015, 15.016, 15.017, 15.019, 15.020, 15.021, 15.022, 15.026, 15.027, 15.033, 15.035, 15.109, 15.113 and 16.027], whereas the five substances [FL-no: 15.005, 15.018, 15.029, 15.030 and 15.032] cannot be evaluated using the Procedure until additional data become available.

For all 21 substances evaluated through the Procedure use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 26 JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests are available for all 26 JECFA-evaluated substances.

Thus, for five substances [FL-no:, 15.005, 15.018, 15.029, 15.030, 15.032,] the Panel could not conclude on their safety when used as flavouring substances as these substances could not be evaluated because of concern with respect to genotoxicity.]..

For the remaining 21 of JECFA-evaluated sulphur-containing heterocyclic compounds [FL-no: 15.001, 15.002, 15.004, 15.008, 15.011, 15.013, 15.014, 15.015, 15.016, 15.017, 15.019, 15.020, 15.021, 15.022, 15.026, 15.027, 15.033, 15.035, 15.109, 15.113 and 16.027] the Panel agrees with the JECFA conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

SUMMARY OF GENOTOXICITY DATA

Table 2: Genotoxicity Data (*in vitro*) for 30 Sulphur-Containing Heterocyclic Compounds Evaluated by the JECFA (JECFA, 2003)

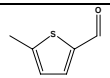
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
1032	Thiazole		Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (8513 µg/plate)	Positive in TA100, negative in TA98; (±S9)	(Lee et al., 1994)
			Ames assay (plate incorporation method)	*S. typhimurium* TA1535; TA1537; TA1538; TA98; TA100	Up to 10000 µg/plate	Negative (±S9)	(Cameron et al., 1985)
			Mouse lymphoma assay	*Mouse L5178Y TK +/-*	1 - 6 µg/ml	Negative (±S9)	(Cameron et al., 1985)
15.017 1035	4,5-Dimethylthiazole		Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (11318 µg/plate)	Negative (±S9)	(Lee et al., 1994)
15.035 1043	4-Methylthiazole		Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9916 µg/plate)	Negative (±S9)	(Lee et al., 1994)
15.004 1050	5-Methyl-2-thiophenecarbaldehyde		Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (12,618 µg/plate)	Negative (±S9)	(Lee et al., 1994)

Table 3: Genotoxicity Data (*in vitro*) EFSA / FGE.21Rev3 (EFSA CEF Panel, 2012) (substances in brackets are JECFA-evaluated substances)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
Subgroup A-Ia						
Thiophene [15.106]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (all strains) (252 µg/plate)	Negative (±S9)	(Florin et al., 1980)	Published non-GLP study. Qualitative screening in a spot-test with three strains, quantitative study (4 doses, 0.03, 0.3, 3, 30 µmol/plate) with TA100 only. Limited report of experimental details and results. Insufficient quality, study not considered adequate for the evaluation of mutagenic activity.
	Ames assay (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate	Negative (±S9)[1]	(Zeiger et al., 1987)	Non-GLP study roughly in accordance with OECD Guideline 471. The study is considered valid.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.01 - 1.2 mmol/plate (100,968 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by ”mutation factor,” no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (8414 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 78.1, 156, 313, 625, 1250 µg/plate	Negative (±S9)	(Shibuya, 2006)	Valid study according to OECD Test Guidelines and Guidelines for screening mutagenicity testing of chemicals (Japan), provided as a translation of the original report in Japanese.
		E. coli WP2 uvrA	0, 78.1, 156, 313, 625, 1250, 2500, 5000 µg/plate	Negative (±S9)		
	Chromosomal Abberation	Chinese hamster lung cells	0, 210, 420, 840 µg/ml	Negative (±S9)	(Tanaka, 2006)	Valid study according to Guidelines for screening mutagenicity testing of chemicals (Japan), provided as a translation of the original report in Japanese.
Subgroup A-Ib						
2-Methylthiophene [15.091]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.00001 - 1.0 mmol/plate (98,170 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by ”mutation factor,” no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.

Table 3: Genotoxicity Data (*in vitro*) EFSA / FGE.21Rev3 (EFSA CEF Panel, 2012) (substances in brackets are JECFA-evaluated substances)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9817 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
3-Methylthiophene [15.092]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.01 - 1.0 mmol/plate (98170 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9817 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
2,5-Dimethylthiophene [15.064]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (11219 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
2-Acetylthiophene [15.040]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (12618 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
2-Acetyl-3-Methylthiophene [15.037]	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
Thiophene-2-carbaldehyde [15.107]	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
5-Ethylthiophene-2-carbaldehyde [15.074]	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
(5-Methyl-2-thiophenecarbaldehyde [15.004])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (12618 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
Subgroup A-II						
2,4-Dimethylthiazole [15.062]	Ames assay (plate incorporation method)	*S. typhimurium* TA100	9.3 and 94 mmol/l top agar (10639 µg/ml)	Negative (-S9)	(Voogd et al., 1983)	Insufficient quality (one test strain as well as without metabolic activation only).
(4,5-Dimethylthiazole [15.017])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (11318 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
(4-Methylthiazole	Ames assay	*S. typhimurium*	Up to 100 µmol/plate	Negative	(Lee et al., 1994)	Only two strains used but otherwise acceptable

Table 3: Genotoxicity Data (*in vitro*) EFSA / FGE.21Rev3 (EFSA CEF Panel, 2012) (substances in brackets are JECFA-evaluated substances)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
[15.035])	(plate incorporation method)	TA98; TA100	(9916 µg/plate)	(±S9)		study.
Subgroup A-III						
2-Methyl-4,5-benzothiazole [15.088]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA102; TA1535; TA1537	100 - 10000 µg/plate	Negative (±S9)[1]	(Longfellow, 1998)	Summary report of NCI-short-term test program, results not given in detail.
(Benzothiazole [15.016])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	Up to 5000 µg/plate	Negative (±S9)	(Bayer, 1991)	Summary in IUCLID data set only. According to this summary, the assay was in compliance with GLP; accordance with OECD Guideline 471 not stated.
	Mouse lymphoma assay	Mouse L5178Y tk+/- cells	10 - 250 µg/ml	Negative (±S9)	(Longfellow, 1997)	Summary report of NCI-short-term test program, results not given in detail.
Subgroup B-III						
2-Propylthiazolidine [15.099]	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA98 and TA100.(±S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
2-Methylthiazolidine [15.090]	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100; (±S9) 100 µg/ml: positive in TA98 and TA100 (±S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Ethylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA100 (±S9) and TA98 (-S9); 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive TA98 and TA100.(±S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Isopropylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA100 (±S9) and TA98 (-S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Butylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA100 (+S9); 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA100 (±S9) and TA98	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).

Table 3: Genotoxicity Data (*in vitro*) EFSA / FGE.21Rev3 (EFSA CEF Panel, 2012) (substances in brackets are JECFA-evaluated substances)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
				(-S9)		
(2-Isobutylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA98 and TA100 (+S9); 10 µg/ml: positive in TA98 and TA100 (±S9); 100 µg/ml: positive in TA98 and TA100 (±S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).

NR: Not Reported
1: With and without rat and hamster S9 metabolic activation.

No *in vivo* mutagenicity/genotoxicity data are available for the candidate substance of the present Flavouring Group Evaluation nor for the supporting substances evaluated by the JECFA at the 59th meeting.

Table 4: Genotoxicity Data (*in vitro*). Summary of Additionally Genotoxicity Data on [FL-no: 15.004] of Subgroup 5.2 of FGE.19

FL-no	Chemical Name	Test System *in vitro*	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
[15.004]	5-Methyl-2-thiophenecarbaldehyde	Reverse Mutation	*S. typhimurium* TA98, TA1535, TA1537 and TA102	0.32-1000 µg/plate [1,2];	Negative	(Beevers, 2009)	Valid study performed in accordance with OECD Guideline 471 and in compliance with GLP.
			S. typhimurium TA100	1.6-5000 µg/plate [1,2]			
			S. typhimurium TA98*a*, TA100*b*, TA102*c*, TA1535*d*, and TA1537*e*	10.24-1000 µg/plate [2,4,*a*,*c*,*d*,*e*]; 10.24-1000 µg/plate [3,5,*a*,*d*]; 25.6-2500 µg/plate [2,4,*d*,*e*]; 4.096-400 µg/plate [3,5,*b*,*c*,*e*]	Negative		
			S. typhimurium TA100*a*, TA1535*b*, TA1537*c*	156.25-5000 ug/plate [2,5,*a*,*b*]; 156.25-5000 ug/plate [2,4,*a*,*b*,*c*];	Negative		
		Micronucleus Assay	Human peripheral blood lymphocytes (Female and Male Donors)	600-1000 µg/ml [4,6]; 50-70 µg/mL [5,6]; 120-350 µg/mL [4,7]; 50-80 µg/mL [5,6]	Weak positive +S9.	(Lloyd, 2011)	Valid study performed in accordance with OECD Guideline 471 and in compliance with GLP.

[1] With and without S9 metabolic activation.
[2] Plate incorporation method.
[3] Pre-incubation method.
[4] Without S9 metabolic activation.
[5] With S9 metabolic activation.
[6] 3-hour incubation with 21-hour recovery period.
[7] 24-hour incubation with no recovery period.

Table 5: Genotoxicity Data (*in vivo*). Summary of Additionally Genotoxicity Data on [FL-no: 15.004] of Subgroup 5.2 of FGE.19

FL-no	Chemical Name	Test System *in vivo*	Test Object / Administration	Concentrations of Substance and Test Conditions	Result	Reference	Comments
[15.004]	5-Methyl-2-thiophenecarbaldehyde	Micronucleus Assay in rat bone marrow	Han Wistar rats (F+M) / Gavage	70, 350 and 700 mg/kg bw/day (males only)	Negative	(Beevers, 2012)	Valid study. In accordance with draft OECD Guideline 474 (2012), and in compliance with GLP. Top dose was the maximum tolerated. Systemic exposure indicated by liver function changes.
		Comet assay in rat liver	Han Wistar rats (F+M) / Gavage	70, 350 and 700 mg/kg bw/day (males only)	Negative	(Beevers, 2012)	The study is in compliance with international accepted guidelines and in compliance with GLP. Top dose was maximum tolerated. Exposure to target organ indicated by liver function changes.

SUMMARY OF SAFETY EVALUATIONS

Table 6: Summary of Safety Evaluation of Sulphur-Containing Heterocyclic Compounds (JECFA, 2003)

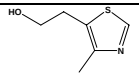
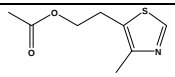
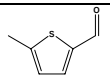
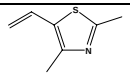
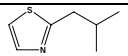
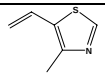
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
15.014 1031	5-(2-Hydroxyethyl)-4-methylthiazole		150 380	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.015 1054	4-Methyl-5-(2-acetoxyethyl)thiazole		8.6 3	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.004 1050	5-Methyl-2-thiophenecarbaldehyde		0.73 0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Evaluated in FGE.224, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.005 1039	2,4-Dimethyl-5-vinylthiazole		0.012 0.007	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Genotoxicity data required.	
15.013 1034	2-Isobutylthiazole		2.3 0.4	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.017 1035	4,5-Dimethylthiazole		0.18 0.4	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.018 1038	4-Methyl-5-vinylthiazole		2.1 0.2	Class II B3: Intake below threshold, B4: Adequate NOAEL	4)	Genotoxicity data required.	

Table 6: Summary of Safety Evaluation of Sulphur-Containing Heterocyclic Compounds (JECFA, 2003)

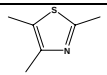
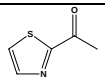
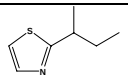
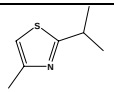
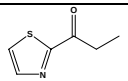
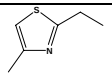
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
				exists			
15.019 1036	2,4,5-Trimethylthiazole		0.61 0.3	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.020 1041	2-Acetylthiazole		9.7 10	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.022 1033	2-(sec-Butyl)thiazole		0.024 0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.026 1037	2-Isopropyl-4-methylthiazole		19 10	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.027 1042	2-Propionylthiazole		0.056 0.2	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.028 1032	Thiazole		0.012 0.07	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No longer supported by Industry (EFSA, 2011).	No longer supported by Industry (EFSA, 2011).
15.033 1044	2-Ethyl 4-methylthiazole		3.2 1	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.035 1043	4-Methylthiazole		0.097 0.05	Class II B3: Intake below threshold,	4)	No safety concern at the estimated level of intake based on the MSDI	No safety concern at the estimated level of intake based on the MSDI

Table 6: Summary of Safety Evaluation of Sulphur-Containing Heterocyclic Compounds (JECFA, 2003)

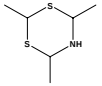
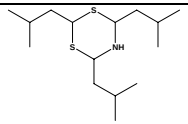
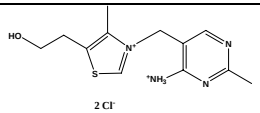
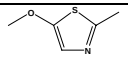
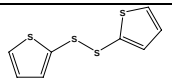
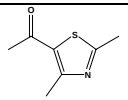
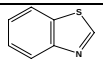
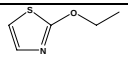
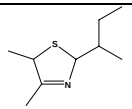
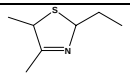
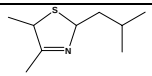
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
				B4: Adequate NOAEL exists		approach.	approach.
15.109 1049	2,4,6-Trimethyldihydro-1,3,5(4*H*)-dithiazine		1.1 3.3	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.113 1048	5,6-Dihydro-2,4,6,tris(2-methylpropyl)-4*H*-1,3,5-dithiazine		2.4 2.6	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.027 1030	Thiamine hydrochloride		300 1200	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.001 1052	2-Mercaptothiophene		0.012 0.03	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.002 1057	2-Methyl-5-methoxythiazole		0.012 0.01	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.008 1053	2-Thienyl disulfide		0.061 0.07	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.011 1055	5-Acetyl-2,4-dimethylthiazole		0.012 2	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

Table 6: Summary of Safety Evaluation of Sulphur-Containing Heterocyclic Compounds (JECFA, 2003)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
15.016 1040	Benzothiazole		1.2 0.2	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.021 1056	2-Ethoxythiazole		0.012 0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.029 1059	2-(sec-Butyl)-4,5-dimethyl-3-thiazoline		0.012 5	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Genotoxicity data required.	
15.030 1058	4,5-Dimethyl-2-ethyl-3-thiazoline		0.012 0.01	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Genotoxicity data required.	
15.032 1045	4,5-Dimethyl-2-isobutyl-3-thiazoline		0.012 4	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Genotoxicity data required.	

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.

Table 7: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA/FGE.21Rev3) (EFSA CEF Panel, 2012)

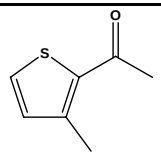
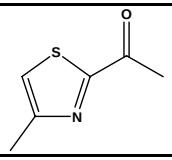
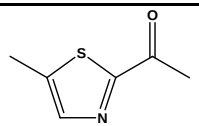
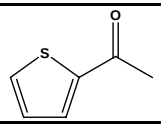
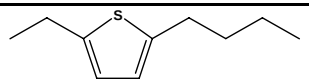
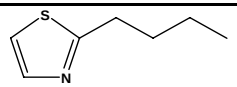
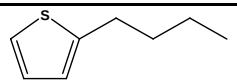
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.037	2-Acetyl-3-methylthiophene		0.18	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.038	2-Acetyl-4-methylthiazole		0.0049	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.039	2-Acetyl-5-methylthiazole		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.040	2-Acetylthiophene		2.2	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.043	2-Butyl-5-ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.044	2-Butylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.045	2-Butylthiophene		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

Table 7: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA/FGE.21Rev3) (EFSA CEF Panel, 2012)

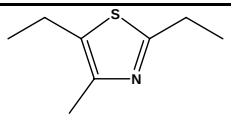
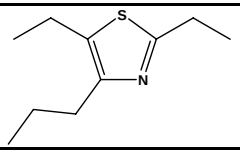
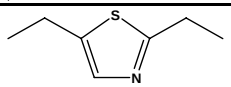
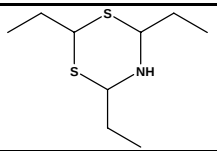
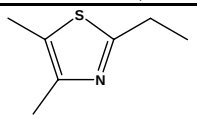
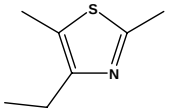
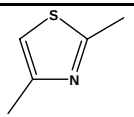
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.050	2,5-Diethyl-4-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.051	2,5-Diethyl-4-propylthiazole		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.052	2,5-Diethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.054	Dihydro-2,4,6-triethyl-1,3,5(4*H*)-dithiazine		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.058	4,5-Dimethyl-2-ethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.061	2,5-Dimethyl-4-ethylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.062	2,4-Dimethylthiazole		0.61	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 7: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA/FGE.21Rev3) (EFSA CEF Panel, 2012)

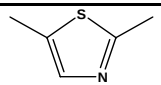
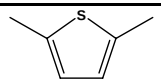
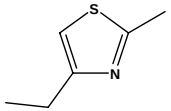
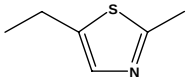
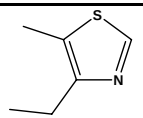
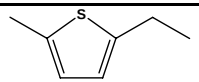
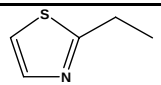
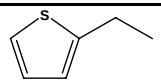
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.063	2,5-Dimethylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.064	2,5-Dimethylthiophene		0.23	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.067	4-Ethyl-2-methylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.068	5-Ethyl-2-methylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.069	4-Ethyl-5-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.070	2-Ethyl-5-methylthiophene		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.071	2-Ethylthiazole		0.028	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.072	2-Ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

Table 7: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA/FGE.21Rev3) (EFSA CEF Panel, 2012)

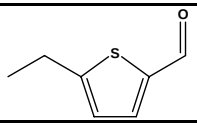
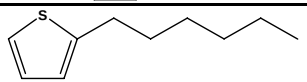
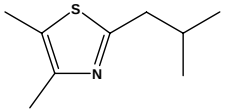
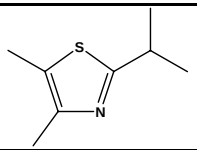
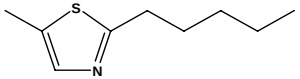
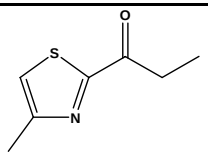
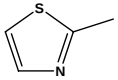
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.074	5-Ethylthiophene-2-carbaldehyde		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.076	2-Hexylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.078	2-Isobutyl-4,5-dimethylthiazole		0.12	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.080	2-Isopropyl-4,5-dimethylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.084	5-Methyl-2-pentylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.085	4-Methyl-2-propionylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.089	2-Methylthiazole		0.018	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 7: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA/FGE.21Rev3) (EFSA CEF Panel, 2012)

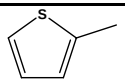
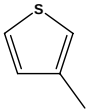
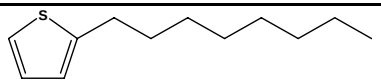
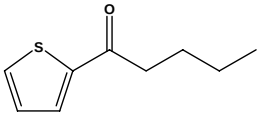
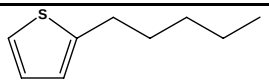
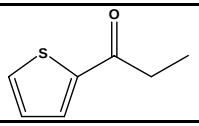
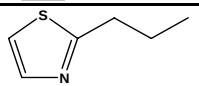
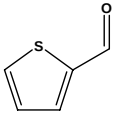
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.091	2-Methylthiophene		0.019	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.092	3-Methylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.093	2-Octylthiophene		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.094	2-Pentanoylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.096	sec-Pentylthiophene		0.24	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.097	2-Propionylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.098	2-Propylthiazole		0.085	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.107	Thiophene-2-carbaldehyde		0.21	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)

Table 7: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA/FGE.21Rev3) (EFSA CEF Panel, 2012)

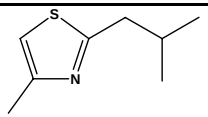
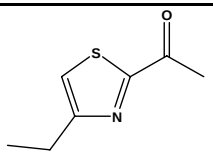
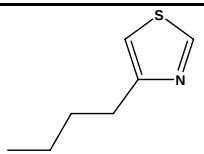
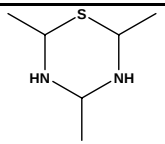
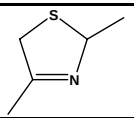
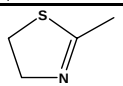
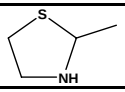
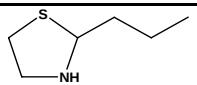
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.115	2-Isobutyl-4-methyl thiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.116	2-Acetyl-4-ethylthiazole		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.118	4-Butylthiazole		1.3	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine		0.61	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.060	2,4-Dimethyl-3-thiazoline		0.012	Class II No evaluation			b)
15.086	2-Methyl-2-thiazoline		0.24	Class II No evaluation			b)
15.090	2-Methylthiazolidine		0.024	Class II No evaluation			c)
15.099	2-Propylthiazolidine		0.012	Class II No evaluation			c)

Table 7: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA/FGE.21Rev3) (EFSA CEF Panel, 2012)

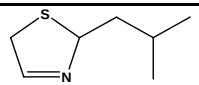
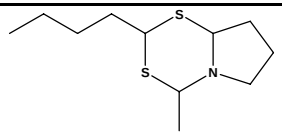
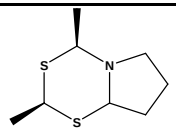
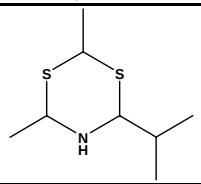
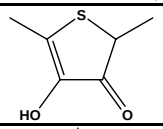
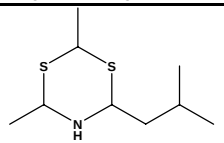
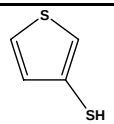
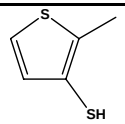
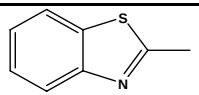
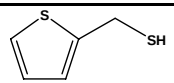
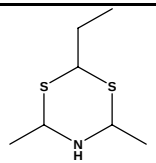
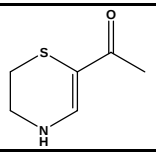
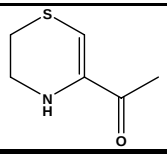
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.119	2-Isobutyl-3-thiazoline		0.011	Class II No evaluation			b)
15.042	2-Butyl-4-methyl(4*H*)pyrrolidino[1,2 d]-1,3,5-dithiazine		0.0012	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.055	2,4-Dimethyl(4*H*)pyrrolidino[1,2e]-1,3,5-dithiazine		0.055	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine		1.5	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine		5.7	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.082	3-Mercaptothiophene		0.011	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 7: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA/FGE.21Rev3) (EFSA CEF Panel, 2012)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.087	2-Methyl-3-mercaptothiophene		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.088	2-Methyl-4,5-benzothiazole		0.0085	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.106	Thiophene		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.108	2-Thiophenemethanethiol		0.0073	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.135	Ethyl thialdine		0.61	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.114	5-Acetyl-2,3-dihydro-1,4-thiazine		0.012	Class III No evaluation			c)
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine		0.61	Class III No evaluation			c)

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.
6) No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.
a) Substance not supported by Industry (EFFA, 2009).
b) Genotoxic potential *in vitro*.
c) Genotoxic potential *in vitro*. Substance not supported by Industry (EFFA, 2009).

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ABBREVIATIONS

BW	body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
DNA	deoxyribonucleic acid
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	good laboratory practice
ID	identity
IR	infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MNBN	micronucleated binucleate cells
MSDI	maximised survey-derived daily intake
mTAMDI	modified theoretical added maximum daily intake
MTD	maximum tolerated dose
NCE	normochromatic erythrocyte
No	number
NOAEL	no observed adverse effect level
OECD	Organization for Economic Cooperation and Development
PCE	polychromatic erythrocyte
RI	replication index
SCF	Scientific Committee on Food

WHO	World Health Organisation